

Table 2

Average group intra-assay %CV for Sample Preparation Method.

		Neat	Digested	2xDilution	4xDilution
Luminex Polystyrene (n=16)	IL6	22.4 (IQR 18.8–29.9) (n=7)	6.1 (IQR 3.8–13.7) (n=15)		
	IL8	26.5 (SD 16.3) (n=7)	4.8 (SD 1.8) (n=13)		
Luminex Magnetic (n=7)	IL6	28.2 (SD 9.4)	6.7 (SD 6.0)	26.0 (SD 16.5)	20.8 (SD 16.6)
Meso Scale Discovery (n=7)	IL6	6.6 (SD 2.5)	7.2 (SD 8.0)	13.3 (9.0)	14.2 (SD 13.0)
	IL8	10.2 (SD 6.8)	10.9 (SD 6.2)	15.2 (SD 9.6)	15.8 (SD 13.2)
	TNF- α	25.4 (SD 14.2)	14.5 (SD 5.9)	18.8 (SD 19.4)	27.0 (SD 16.4)
	IL1 β	55.3 (SD 37.6)	69.8 (SD 27.9)	67.6 (SD 29.0)	124.1 (SD 57.1)

recommend sample pre-treatment with 2mg/ml hyaluronidase (& 2-fold dilution) for multiplex synovial fluid analysis.

156**A WIDE ASSOCIATION STUDY OF PREDICTORS OF FUTURE KNEE PAIN: DATA FROM THE OSTEOARTHRITIS INITIATIVE**

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Purpose: Pain is a common symptom of knee OA. The presentation of the symptom is protean, and varies from person to person and from disease stage to disease stage. WOMAC pain sub scores assess a subject's pain while: 1) In bed; 2) Walking; 3) Climbing stairs; 4) Standing; and 5) Lying down. These five categories reflect, presumably, different etiologies of pain. To better understand the etiology of pain in these settings, the OAI has been capturing thousands of variables (variable is a specific clinical data) in OA patients; hopefully, evaluation of these variables will allow a better understanding of OA, and establish predictors of OA related pain. The objective of this work was to use automated wide association tools to determine which OAI variables, singularly or in association with other variables, best predict future OA related pain.

Methods: The 4786 OAI enrolled subjects and the 1226 variables of the OAI baseline clinical datasets (OAI:0.2.2) and the 36 month WOMAC pain sub-score data (OAI:5.2.1) were explored in this study. Baseline variables with more than 10% of missing information were removed. Subjects with 36 month missing WOMAC pain data were removed. Therefore, only 4204 patients and 629 variables were included in this study. Furthermore, to explore the no-pain related sources of future knee pain all baseline pain related variables were removed. The max value from the left and right knee pain sub-scores were used as the outcome variable to be predicted by a five variable multidimensional nearest centroid classifier using BIOMATEC (Monterrey, Mexico). The top predictor variables of each pain sub-score were selected after generating 5000 models using a 33%, 67% split between training and testing sets. Once the top variables were selected, linear models and R software were used to get the significance of those variables in predicting the 36 month WOMAC pain scores.

Results: All linear regression models were highly significant ($p < 1.0 \times 10^{-10}$) with R^2 ranging from 0.199 to 0.328. Left knee catch or hand up when moving, folate supplements, having a symptomatic knee, physical scale and right knee stiffness score predicted pain while lying down. Health limiting moderate activities, WOMAC knee stiffness, left knee KOOS symptoms and blood pressure predicted pain in Bed. Climbing stairs pain was predicted by lack of confidence in knees and WOMAC disability score. Health limiting moderate activities, left knee swelling, lack of confidence in knees and WOMAC stiffness score predicted pain while walking. Standing pain was predicted by KOOS symptom score and 400 meters walk time physical test.

Conclusions: Wide association studies can be very helpful in data mining the OAI databases. These tools were used to explore models predictive of the 36 month pain sub scores and yielded highly predicting models. Therefore, three year pain prognosis is possible using clinical data.

RESULTS			
Kind of pain	Model	P value	R-squared
Pain in bed	A48, A60, A73, A77, A448	p-value < 2.2e-16	0.1985
Pain Lie Down	A65, A71, A73, A80, A377	p-value < 2.2e-16	0.2094
Pain stairs	A49, A70, A75, A80, A81	p-value < 2.2e-16	0.3279
Pain stand	A48, A75, A77, A80, A549	p-value < 2.2e-16	0.3167
Pain walking	A48, A63, A70, A73, A80	p-value < 2.2e-16	0.259
Variable description in order of relevance based on model appearance frequency			
A80: Baseline symptomatic knee OA status by person (calc, used for study elig (>0) and in OAI definition of cohort status)			
A73: Right knee: WOMAC Stiffness Score (calc)			
A48: How much health limit involvement in moderate activities (e.g., moving a table, pushing vacuum cleaner)			
75: Right knee: WOMAC Disability Score (calc)			
A77: Left knee: KOOS Symptoms Score (calc)			
A70: Quality of life: how much troubled with lack of confidence in knee(s)			
A49: How much health limit climbing several flights of stairs			
A60: Right knee symptoms: knee catch or hang up			
A63: Left knee symptoms: swelling, last 7 days			
A65: Left knee symptoms: knee catch or hang up when moving, last 7 days			
A71: Physical summary scale for the MOS 12-item short-form health survey (SF-12) v2 (calc)			
A81: Left knee: WOMAC Total Score (calc)			
A377: Average daily nutrients from vitamin supplements, folate (mcg) (calc)			
A448: Blood pressure systolic (mm Hg)			
A549: 400-meter walk: total time at 400-m or at stop (sec) (calc)			

157**URINARY CTX-II IS MORE USEFUL IN POSTMENOPAUSAL WOMEN THAN IN MEN TO DETECT RADIOLOGICAL KNEE OA: THE MATSUDAI KNEE OSTEOARTHRITIS SURVEY**

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Purpose: Biochemical markers of cartilage degradation are important indices to evaluate knee osteoarthritis (OA). The correlation between radiological knee OA and urine CTX-II (C-terminal crosslinking telopeptide of collagen type II: uCTX-II) needs to be evaluated how this marker is useful in the health check-up in a large population-based study.

Methods: We have performed the epidemiological knee survey in a rural Japanese population at the Matsudai district in Niigata Prefecture, Japan since 1979. In 2010 (6th survey), a cross-sectional study of biomarkers was conducted with informed consent. Urine specimens were collected from 942 subjects, and uCTX-II was measured using Urine CartiLaps EIA. The data more than 1000 ng/mmmol Cre were considered as outliers. Subjects with oral administration of bisphosphonate, fracture within a year, or TKA were excluded. Standing knee AP X-rays were obtained and graded according to the Kellgren-Lawrence (K-L) classification. The 60- to 79-year-old subjects were selected and then divided by gender (260 female and 276 male) and OA grade (K-L Grade 0,1, Grade 2, and Grade 3,4 groups). The levels of uCTX-II were compared between three OA grade groups using Kruskal-Wallis test and Mann-Whitney U-test. The subjects were stratified into quartiles based on the values of uCTX-II, and proportion of OA grades in each quartile was compared using chi-square test. In addition, to

estimate the predictive value of uCTX-II, ROC curve analysis was employed and area under the curve (AUC) was evaluated. $p < 0.05$ was considered statistically significant.

Results: In female subjects, uCTX-II values significantly increased according to the severity of knee OA (Fig.1A). In male subjects, OA G3,4 group had significantly higher uCTX-II values than G0,1 group or G2 group (Fig.1B).

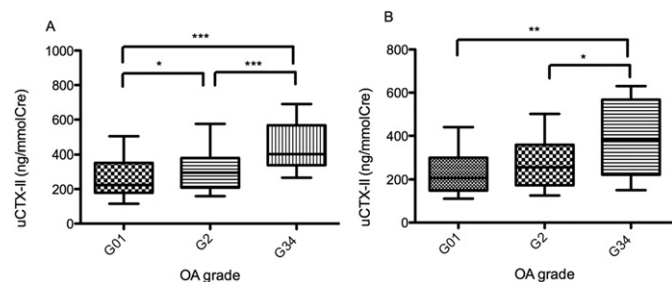


Fig 1. Relationship between knee X-ray OA grades and uCTX-II levels in (A) female and (B) male subjects. Each box represents the 25th/50th (median) to 75th percentiles. The lines outside the box represent the 10th and 90th percentiles. *, $P < 0.05$. **, $P < 0.01$, ***, $P < 0.001$.

The mean levels of uCTX-II in each quartile were 152, 236, 345, 554 ng/mmol Cre in female and 121, 188, 269, 469 in male. Higher quartiles (Q3 and Q4) of uCTX-II level included higher numbers of OA ($\geq G2$) subjects in female but not significant in male (Fig.2).

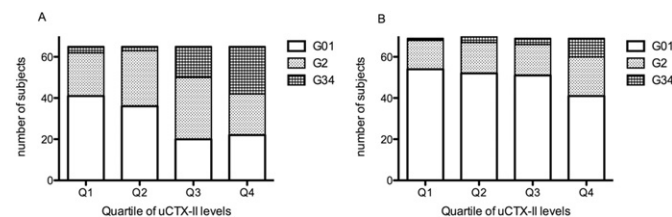


Fig. 2 Quartiles by uCTX-II levels and proportion of OA grades in (A) female and (B) male. Each quartile has 65 and 69 subjects in female and male. Significant difference was observed only in female ($p < 0.0001$, Chi-square). ROC curve analysis for discriminating $\geq G2$ OA showed that AUC was 0.65 and 0.61 in female and male, respectively. If cut-off is 300 in female, sensitivity and specificity are 60 and 65 %, respectively.

Conclusions: uCTX-II is more useful in female older than 60 years but a modest biomarker of knee OA X-ray grade by the single measurement in the cross-sectional study. OA changes of other joints (e.g. lumbar spondylosis) may affect the level of uCTX-II. Further analysis of longitudinal changes of uCTX-II and knee OA grade using the data of 2007 (5th) and 2010 surveys is in progress.

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PERFORMANCE METRICS OF A NEWLY FORMATTED TYPE II COLLAGEN CLEAVAGE NEO-EPITOPE ASSAY FOR HUMAN URINE: C2C-HUSA

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Purpose: The purpose of this study was to evaluate the performance metrics of a new C2C sandwich immunoassay (IB-C2C-HUSA), including reproducibility and linearity as well as variation due to physical activity or food consumption, to inform biospecimen collection protocols in clinical trials. Type II collagen is the most abundant collagen in articular cartilage, providing tensile strength, and is cleaved by collagenases, resulting in the creation of a neopeptide at the C terminus of the $3/4$ length fragment, detectable in human body fluids by the C2C competitive ELISA. Once this initial cleavage has occurred, proteases create subsequent degradation products containing the C-terminal neopeptide that are detectable in human urine.

Methods: Forty participants with knee OA (20 patients for each of two cohorts) were admitted overnight for serial urine sampling to assess the variation of OA-related biomarkers. Urine samples were obtained on the

evening (6-8PM) of Day 1 (T3; n=34), prior to rising (8AM) from bed (T0; n=34), 1 hr after rising (9AM) without food consumption (T1a; n=20), 1-2 hr after rising (9-10AM) with food consumption (T1; n=34), and at noon, 4 hr after rising (T2; n=14). Urine C2C was measured using the new IB-C2C-HUSA and values were corrected for creatinine concentrations and reported as ng/mmol Cr. The linearity of the assay was determined by testing each urine sample at neat, 1:2, and 1:4 dilutions. The percent recovery was calculated as the adjusted concentration versus the adjusted concentration of the previous dilution. The acceptance criteria for linearity are between 70–130%. To assess for variation due to activity and food consumption across the cohorts, the biomarker concentrations at each time point for each subject were normalized to the mean concentration of the four time points for that individual. Results were analyzed using non-parametric Friedman's test with Dunn's post-hoc multiple comparison test. To compare the results of the newly formatted assay to the original C2C assay, correlation analyses of the IB-C2C-HUSA to C2C values measured previously in the same samples was performed. Statistical analyses were performed using GraphPad software and a p-value < 0.05 was considered significant.

Results: C2C was measurable in all urine samples using the new IB-C2C-HUSA and values from analysis of neat samples were used for 98.5 percent of the determinations. For samples that had concentrations higher than the highest standard (5000 pg/ml), a dilution factor of 1:2 was used to determine the final concentration. The standard curve was linear ($R^2 = 0.992$) and the assay was linear from neat to 1:4 dilutions with a mean percent recovery of 126%. The mean concentrations \pm SD and range for the IB-C2C-HUSA biomarker at each time point (pg/ml) were as follows: T0: 1244 \pm 1055 (249–5246); T1a: 1635 \pm 1205 (344–5771); T1: 1871 \pm 1032 (443–4952); T2: 1074 \pm (239–3213); T3: 1043 \pm 814 (240–3845). Whereas C2C concentrations, uncorrected for urine creatinine, varied greatly within a day (Fig 1A), C2C concentrations corrected for creatinine demonstrated no statistically significant variation of the biomarker with either activity (T1a) or with food consumption (T1) (Fig 1B), the two conditions most likely to affect sampling in a clinical trial. A comparison of values obtained from the original C2C and the IB-C2C-HUSA assay at each time point revealed a significant correlation between the original and new formats for T1a ($p = 0.001$, $r^2 = 0.46$), T1 ($p = 0.04$, $r^2 = 0.12$), and T2 ($p = 0.03$, $r^2 = 0.33$).

Conclusions: The new IB-C2C-HUSA immunoassay is a highly reproducible sandwich assay that yielded measurable type II collagen neopeptide fragments in all 136 samples tested. Compared with the original C2C competitive ELISA, this new sandwich ELISA did not show variation due to activity. This makes it useful for the measurement of collagen degradation in OA studies, and in particular, it would be appropriate for analyses of legacy samples, obtained without regard to standardization of time of collection.

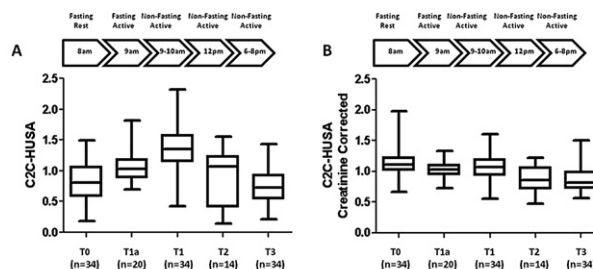


Figure 1. Comparison of the variation throughout the day of C2C-HUSA with and without creatinine correction. The median values with interquartile ranges (and maximum and minimum values) are shown in the box plots of normalized concentrations. Normalization was achieved by dividing each biomarker concentration by the mean concentration of the four time points for that individual.

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ELEVATED EXPRESSION OF MMP9 IN PLASMA OF PATIENTS WITH SYMPTOMATIC KNEE OSTEOARTHRITIC: CORRELATION WITH DISEASE SEVERITY AND PROGRESSION

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